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Soluble CD93 Is Involved in Metabolic Dysregulation but Does Not Influence Carotid Intima-Media Thickness

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METABOLISM

Type 2 diabetes and cardiovascular disease are complex disorders involving metabolic and inflammatory mechanisms. Here we investigated whether sCD93, a group XIV c-type lectin of the endosialin family, plays a role in metabolic dysregulation or carotid intima-media thickness (IMT). Although no association was observed between sCD93 and IMT, sCD93 levels were significantly lower in subjects with type 2 diabetes (*n* = 901, mean ± SD 156.6 ± 40.0 ng/mL) compared with subjects without diabetes (*n* = 2,470, 164.1 ± 44.8 ng/mL, *P* < 0.0001). Genetic variants associated with diabetes risk (DIAGRAM Consortium) did not influence sCD93 levels (individually or combined in a single nucleotide polymorphism score). In a prospective cohort, lower sCD93 levels preceded the development of diabetes. Consistent with this, a *cd93*-deficient mouse model (in addition to *apoe* deficiency) demonstrated no difference in atherosclerotic lesion development compared with *apoe*$^{-/-}$ *cd93*-sufficient littermates. However, *cd93*-deficient mice showed impaired glucose clearance and insulin sensitivity (compared with littermate controls) after eating a high-fat diet. The expression of cd93 was observed in pancreatic islets, and leaky vessels were apparent in *cd93*-deficient pancreases. We further demonstrated that stress-induced release of sCD93 is impaired by hyperglycemia. Therefore, we propose CD93 as an important component in glucometabolic regulation.

Subjects with type 2 diabetes (T2D) have a twofold to fourfold greater risk for the development of cardiovascular disease (CVD) than those without. Preventive strategies targeting CVD have shown little progress in subjects with

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T2D, despite their efficacy in subjects without diabetes. Although complete understanding of the mechanisms leading to CVD is lacking, a combination of metabolic dysregulation and inflammatory pathways are important contributors. Therefore, elucidation of pathways linking metabolic dysregulation and inflammation could pinpoint potential therapeutic targets for reducing CVD, especially in subjects with T2D.

CD93 is a group XIV c-type lectin belonging to the endosialin family, originally described as a component of the complement system (1). CD93 is composed of a cytoplasmic tail containing a PDZ binding domain (2), a transmembrane domain containing metalloproteinase sites, an extracellular region containing a mucin-like domain that is highly glycosylated, five epidermal growth factor domains (four in mice), and a unique C-type lectin domain. CD93 is predominantly expressed on endothelial cells, but also in innate immune cells such as neutrophils and monocytes as well as in megakaryocytes (3). In response to certain inflammatory molecules, the transmembrane CD93 is cleaved and the extracellular segment is released into the circulation as soluble CD93 (sCD93) (4,5). It is still unknown whether the released sCD93 has a distinct function, or whether the release of this fragment is merely to enable the intracellular remnant to respond to the cellular stress. Described as a factor involved in the removal of apoptotic bodies, CD93 has also been involved in B-cell maturation and natural killer T (iNKT) cell survival (6). Epidermal growth factor domains are believed to be involved in angiogenesis (7), and the moesin-binding domain (8) is required for endothelial cell-cell interactions (9).

Regarding metabolism and CVD, *CD93* is a plausible candidate in the mouse nonobese diabetes *Idd13* locus (10), and we have previously shown that reduced levels of circulating sCD93 are associated with increased risk of myocardial infarction (11). More recently, the *CD93* gene has been identified as a potential regulator of pathways common to both T2D and CVD (12). Interestingly, *CD93* expression is upregulated by conditions relevant to diabetes or its complications, for example, flow-related shear stress (13) due to endothelial dysfunction; during the development of new but leaky blood vessels (14), as observed in retinopathy; and during ischemia-related inflammation of cerebral vascular endothelium (15), thus reflecting myocardial infarction.

Here we investigated sCD93 for effects on markers of metabolic dysregulation and early CVD in human cohorts and in a mouse model with a genetic deficiency in sCD93. We further examined the mechanisms by which sCD93 acts.

RESEARCH DESIGN AND METHODS

Discovery Analyses: IMPROVE Study

The IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) Study cohort has previously been described (16,17). Briefly, subjects with at least three established CVD risk factors without symptoms or history of coronary artery disease were enrolled in the study from seven European centers (at latitudes ranging from 43° to 62° North). Medical history, anthropometric measurements, and blood samples were obtained at baseline, and standard biochemical phenotyping was performed. Blood samples were stored at −80°C. Extensive carotid intima-media thickness (IMT) phenotyping was performed by ultrasound at baseline, as well as at 15 and 30 months after study enrollment (16,17). Approval was granted by the regional ethics committee for each recruitment center, and written informed consent was provided by all participants. T2D was defined as diagnosis, use of antidiabetic medication, or fasting glucose level ≥7 mmol/L. sCD93 was measured using the Meso Scale platform, using the previously validated ELISA antibodies (11) and SECTOR Imager 2400. Characteristics of the cohort are presented in Table 1.

IMPROVE Study Genotyping

Reported T2D risk-associated single nucleotide polymorphisms (SNPs) (18) were genotyped in the IMPROVE Study cohort using the Illumina Metabochip (19) and Immunochip (20) platforms. Genotyping was conducted at the SNP&SEQ Technology Platform (Uppsala University, Uppsala, Sweden), and standard quality control was conducted. Subject exclusions were as follows: low call rate (<95%), cryptic relatedness, or ambiguous sex. SNP exclusions were as follows: failing call rate (<95%) or Hardy-Weinberg equilibrium ($P < 5*10^{-6}$) thresholds. After quality control, multidimensional scaling (MDS) components were calculated using PLINK (21) with default settings. The first MDS component demonstrates strong correlations with latitude of recruitment center (Spearman rank correlation: $\rho = 0.935$ and $P < 0.0001$ and $\rho = 0.946$ and $P < 0.0001$, respectively, for subjects without and with T2D).

Statistical Analyses: IMPROVE Study Epidemiology

The trend test for ordered groups was used to assess an effect of recruitment center latitude. Differences in sCD93 levels between men/women and subjects with/without diabetes were assessed by *t* test. Associations between sCD93 levels and established risk factors were assessed by Spearman rank correlation coefficients. Skewed variables, including sCD93, were log transformed for further statistical analyses. Multivariable regression analysis was used to identify markers of metabolism or CVD with significant effects on sCD93 levels. Variables considered for study inclusion were as follows: age and sex (forced into the models), height, weight, BMI, waist-to-hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), LDL cholesterol, HDL cholesterol, triglycerides (TGs), fasting glucose, C-reactive protein, proinsulin, insulin, HOMA indices, adiponectin, leptin, interleukin 5 (IL-5), current smoking, and use of lipid-lowering and antihypertensive medication. Multivariable regression, adjusted for established CVD risk markers (age, sex, MDS 1–3, BMI, SBP, HDL, TGs, and current smoking status) (22), was used to assess the effect of sCD93 levels on measures of IMT. Analyses were conducted using STATA version 11.2 (StataCorp, College Station, TX).

Table 1—IMPROVE cohort characteristics

	Without diabetes (*N* = 2,470)	T2D (*N* = 901)	*P*
Male sex, *n* (%)	1,138 (44.7)	533 (57.4)	**<0.0001**
Age (years)	64.2 (5.4)	64.2 (5.6)	0.8246
Height (m)	1.67 (0.09)	1.69 (0.09)	**<0.0001**
BMI (kg/m^2)	26.6 (3.9)	29.2 (4.6)	**<0.0001**
WHR	0.91 (0.08)	0.95 (0.09)	**<0.0001**
SBP	141 (19)	145 (18)	**<0.0001**
DBP	82 (10)	82 (10)	0.3524
LDL (mmol/L)	3.71 (0.97)	3.07 (0.95)	**<0.0001**
HDL (mmol/L)*	1.31 (0.36)	1.14 (0.33)	**<0.0001**
TG (mmol/L)*	1.47 (0.90)	1.91 (1.82)	**<0.0001**
Fasting glucose (mmol/L)*	5.29 (0.67)	7.71 (2.18)	**<0.0001**
C-reactive protein (mmol/L)*	2.89 (6.16)	3.20 (4.22)	**0.0001**
CD93 (ng/mL)*	164 (45)	157 (40)	**<0.0001**
Fasting proinsulin (pmol/L)*	6.03 (6.26)	10.5 (8.88)	**<0.0001**
Fasting insulin (pmol/L)*	44.4 (61.5)	66.5 (88.4)	**<0.0001**
HOMA-B*	68.9 (54.3)	50.8 (50.8)	**<0.0001**
HOMA-IR*	0.83 (1.09)	1.33 (1.66)	**<0.0001**
Uric acid (mmol/L)*	309 (70)	333 (76)	**<0.0001**
Creatinine (mmol/L)*	80.3 (17.7)	82.8 (17.7)	**<0.0001**
Vitamin D (nmol/L)*	50.7 (21.5)	48.2 (20.2)	0.963
Adiponectin (μg/mL)*	14.2 (9.9)	9.43 (7.19)	**<0.0001**
Leptin (ng/mL)*	20.0 (17.0)	21.6 (17.4)	**0.0138**
IL-5 (pg/mL)*	0.67 (1.82)	0.86 (3.50)	**<0.0001**
Smoking (pack-years)	9.84 (16.3)	14.1 (18.6)	**<0.0001**
Current smoking (%)	381 (15.0)	143 (15.4)	0.7483
Lipid-lowering medication (%)	1,268 (49.8)	449 (48.6)	0.542
Antihypertensive medication (%)	1,397 (54.8)	604 (65.0)	**<0.0001**
Baseline			
CC-IMT$_{mean}$*	0.738 (0.141)	0.758 (0.145)	**0.0001**
BIF-IMT$_{mean}$*	1.131 (0.396)	1.190 (0.429)	**0.0002**
IMT$_{mean}$*	0.880 (0.196)	0.918 (0.206)	**<0.0001**
CC-IMT$_{max}$*	1.185 (0.196)	1.225 (0.412)	**0.0035**
BIF-IMT$_{max}$*	1.840 (0.750)	1.954 (0.829)	**0.0004**
IMT$_{max}$*	1.998 (0.792)	2.140 (0.862)	**<0.0001**
IMT$_{mean-max}$*	1.239 (0.292)	1.290 (0.312)	**<0.0001**
Progression			
CC-IMT$_{mean}$	0.008 (0.025)	0.011 (0.034)	**0.0031**
BIF-IMT$_{mean}$	0.032 (0.070)	0.040 (0.087)	**0.0134**
IMT$_{mean}$	0.018 (0.030)	0.022 (0.035)	**0.0007**
CC-IMT$_{max}$	0.013 (0.087)	0.019 (0.113)	0.1385
BIF-IMT$_{max}$	0.047 (0.153)	0.058 (0.178)	0.0700
IMT$_{max}$	0.040 (0.157)	0.056 (0.178)	**0.0145**
IMT$_{mean-max}$	0.162 (0.140)	0.188 (0.155)	**0.0482**
Fastest_progression	0.024 (0.051)	0.028 (0.054)	**<0.0001**

Where values are presented as the mean (SD) for continuous measures and *n* (%) for categorical measures, unless otherwise indicated. T2D was defined as diagnosis, antidiabetic medication, or fasting glucose ≥7 mmol/L; Vitamin D, adjusted for season of blood sampling. All IMTs were measured in millimeters. *P* value was determined by *t* test. Boldface type indicates nominal significance. CC, common carotid; BIF, bifurcation. *log10 transformed prior to analysis.

Statistical Analyses: Genetics

Linear regression analyses assuming an additive genetic model were conducted in PLINK (21) to assess the influence of T2D risk-associated SNPs on sCD93 levels, adjusting for age, sex, and population structure (MDS 1–3). Genotypes of 52 (of 62 known) (18) T2D risk-associated SNPs were

combined in an unweighted SNP score by summing the reported (18) T2D risk-increasing alleles for each subject (thus representing the total burden of genetically determined T2D risk). Only subjects without T2D and with complete genotyping were included in this analysis. The score was tested for influence on levels of sCD93, using a linear regression model as above, in STATA version 11.2 (StataCorp).

Replication Analyses: Stockholm Diabetes Prevention Program

The Stockholm Diabetes Prevention Program (SDPP) is a prospective study of subjects from the Stockholm area, who were 35–55 years of age at baseline (23). Briefly, blood samples were collected; oral glucose tolerance tests, basic clinical phenotyping, and questionnaires were conducted in participants at baseline and after 8–10 years of follow-up. Levels of sCD93 were measured by Meso Scale in baseline samples and in a subset of follow-up samples (Supplementary Fig. 1). Baseline samples were from subjects in whom normal glucose tolerance (NGT) (n = 843), prediabetes (defined as impaired glucose tolerance and/or impaired fasting glucose, n = 326), and T2D (n = 113) were newly diagnosed. Follow-up samples from NGT subjects at baseline were also analyzed. Some subjects remained with NGT (n = 370), whereas other subjects had progressed to prediabetes (n = 314) or T2D (158). The Karolinska Institutets Ethics Committee approved the study, and all subjects gave their informed consent. ANOVA (adjusted for age and sex) was used to compare levels of sCD93 between glucose tolerance groups at baseline or after follow-up. t Tests were used to compare baseline levels of sCD93 from subjects with a diagnosis of NGT and prediabetes or T2D at follow-up. The effect of baseline sCD93 levels on the risk of the development of prediabetes or T2D was assessed using logistic regression, adjusting for age and sex, or age, sex, current smoking status, BMI, and blood pressure medication. Analyses were conducted in STATA version 11.2 (StataCorp).

cd93-Deficient Mice

The *cd93*-deficient mouse was generated by the Trans-NIH Knockout Mouse Project (KOMP) and obtained from the KOMP Repository (www.komp.org). Embryonic stem cells were generated from C57BL/6N mice and kept on the C57BL/6N background. The breeding of the *cd93*-deficient mice did not show a Mendelian ratio, with a very low ratio of homozygous knockout mice observed. However, cd93 heterozygous ($cd93^{+/-}$) mice had half the concentration of sCD93 in the periphery compared with their wild-type ($cd93^{+/+}$) littermates (Supplementary Table 1), rendering this a relevant model to be used in comparisons with human studies, because humans have varying levels of sCD93 (11) rather than a complete absence of sCD93. Therefore, this study focuses on $cd93^{+/+}$ and $cd93^{+/-}$ animals. All mice were bred and kept at the Karolinska Institutet animal facility, with a 12 h light/dark cycle and food and water available ad libitum. All procedures were approved by the regional animal ethics authority.

Characterization *cd93*-Deficient Mice

Mouse scd93 was measured using Meso Scale technology with antibodies directed against murine *cd93* (capture antibody clone 223437, detection antibody BAF1696; R&D Systems, Minneapolis, MN) using EDTA plasma from male mice (n = 8 from each genotype) fed a Western diet for 16 weeks. Expression of *cd93* in the B-cell population (Supplementary Table 1) was determined by flow cytometry. Single-cell suspensions of spleen cells from male mice (n = 8 from each genotype) fed a Western diet for 16 weeks were used. First, Fc receptors were blocked with anti-FcRII and anti-FcRIII (clone 24.G2, an in-house preparation). B cells were stained with anti-mouse CD45R eFluor450 (clone RA3–6B2; eBioscience) and anti-mouse-CD19 conjugated with APC-Cy7 (clone 6D5; BioLegend). The percentages of IgG- and IgM-positive B cells was determined using anti-mouse IgG conjugated with fluorescein isothiocyanate (Poly4060; BioLegend) and anti-mouse-IgM conjugated with allophycocyanin (RMM-1; BioLegend). The expression of cd93 on B cells was determined by anti-mouse cd93 conjugated with phycoerythrin (clone AA4.1; BioLegend) on a Beckman Coulter Gallios Flow Cytometer. The percentage of iNKT cells in the liver was determined using a previously published method (10) with the exception that a violet viability dye (Live/Dead; Life Technologies) was included, using the Beckman Coulter Gallios Flow Cytometer.

Tissue Collection and Assessment of Atherosclerotic Lesions in Mice ($Apoe^{-/-}cd93^{+/+}$ and $Apoe^{-/-}cd93^{+/-}$)

For atherosclerosis studies, mice were crossed into *apoe*-deficient ($apoe^{-/-}$) mice (originally from The Jackson Laboratory) and backcrossed six generations to C57BL/6N. Homozygous for *apoe* deficiency mice, with two copies or one copy of the *cd93* gene ($apoe^{-/-}cd93^{+/+}$ and $apoe^{-/-}cd93^{+/-}$, respectively) were fed a normal rodent diet for 32 weeks, at which point blood was sampled via cardiac puncture. Plasma samples (EDTA) were stored at −80°C. Organs were perfused with sterile PBS, and the descending thoracic aorta was collected into 4% paraformaldehyde. The thoracic aorta was pinned onto a paraffin bed, and en face lipid content was determined by staining with Sudan IV (Sigma-Aldrich). Images were captured using a DC480 camera connected to an MZ6 stereomicroscope (both from Leica). Quantification of the area of all the plaques in a given aortic arch were summed and expressed as the percentage of the total surface area of the aorta using ImageJ software (National Institutes of Health).

Metabolic Studies of Mice ($cd93^{+/+}$ and $cd93^{+/-}$)

For metabolic studies, *cd93*-deficient mice were fed a Western diet (SDS custom diet 21% fat 0.2% cholesterol mixed in standard CRM [p] maintenance diet) for 16 weeks. Glucose and insulin tolerance tests were conducted. After 4 h of fasting, a bolus of glucose (1 g/kg for glucose tolerance test) or insulin (0.75 units/kg for insulin tolerance test) was given by intraperitoneal injection. Blood was sampled from the tail vein at 15, 30, 60, and 120 min.

Pancreatic Morphology in the *cd93*-Deficient Mouse Model (*cd93*$^{+/+}$ vs. *cd93*$^{+/-}$)

Differences in pancreas morphology between genotypes were assessed by immunohistochemistry. Mice were fed a Western diet for 16 weeks prior to removal of the pancreas. Embedding and sectioning of the pancreas as well as rehydration and dehydration of sections were conducted as per standard protocols. Four pancreata were analyzed per genotype. To assess the presence and location of insulin, cd93 and Von Willebrand factor (VWF), sections were boiled for 20 min in Diva Decloaker (Biocare Medical), and sections were treated with 3% hydrogen peroxidase before blocking in 5% goat serum in 1% BSA. Serial sections were stained using antibodies against insulin (guinea pig anti-insulin; Abcam), VWF (rabbit anti-VWF; Abcam), and cd93 (rat anti-cd93; R&D Systems). Of note, the anti-cd93 antibody targets an extracellular epitope, and thus is able to detect cell surface–attached, as well as soluble, cd93. After overnight incubation at 4°C, sections were incubated with biotinylated secondary antibodies (goat anti-guinea pig; Abcam; goat anti-rabbit and rabbit anti-rat; Vector Laboratories) for 1 h at room temperature. Peroxidase-avidin/biotin complex was achieved using Vectastain ABC Elite kit (Vector Laboratories) and detected using Novo Red (Vector Laboratories), as per manufacturer directions, and counterstained with hematoxylin. The numbers of islets were counted in parallel by two researchers, using three to five hematoxylin-eosin–stained sections. The size of insulin-stained islets was measured using ImageJ software (National Institutes of Health). The number of VWF-positive islets and the total number of islets was counted, and the percentage of positive islet staining was calculated (number of VWF-positive islets/total number of islets and multiplied by 100). The pancreas from one *cd93*$^{-/-}$ mouse that was also fed a Western diet for 16 weeks was included to confirm the specificity of the anti-cd93 staining.

Blood Vessel Integrity (*cd93*$^{+/+}$ vs. *cd93*$^{+/-}$)

An in vivo blood vessel permeability assay was performed by intravenous injection of 0.5% Evans Blue stain into anesthetized 4-week-old male mice. After 30 min, mice were euthanized and perfused with PBS. After collection, the pancreata were treated with 50% trichloroacetic acid at a 1:4 ratio (in micrograms per milliliter) and homogenized using Bio-Gen Pro200 (Pro Scientific) for 30 s. The amount of Evans Blue was determined as previously published (24) and was detected using GloMax-Multi with fluorescence 625/660–720 (Promega).

Peripheral Markers of Endothelial Damage (*cd93*$^{+/+}$ vs. *cd93*$^{+/-}$)

Soluble E-selectin and VWF A2 were measured in the plasma of mice fed either a Western diet or a chow diet for 16 weeks. E-selectin was measured using Meso Scale and the DuoKit for E-selectin (R&D Systems) with the addition of SULFO-TAG–labeled streptavidin. VWF A2 was measured using SimpleStep ELISA kit from Abcam as per the manufacturer directions.

Statistical Analysis of Murine Data

The Student *t* test was used to determine statistical significance between two groups. Differences between more than two groups were assessed by one-way ANOVA with post hoc analysis using the Tukey multiple-comparison test. When analyzing repeated glucose metabolism measurements, a two-way ANOVA repeated-measurements test was used to establish significance with post hoc analysis using the Šidák multiple-comparisons test. PRISM (GraphPad Software, San Diego, CA) was used for ANOVA procedures.

Analysis of sCD93 Release From Endothelial Cells

To assess the impact of diabetes-relevant conditions on sCD93 release, the human carotid endothelial cells (HCtAECs) in complete endothelial cell growth media (Cell Applications) and human endothelial hybrid cell (EA.Hy 926; ATCC, in RPMI medium, 10% fetal calf serum, and 1% penicillin and streptomycin; Sigma-Aldrich) were expanded in flasks coated with gelatin (Sigma-Aldrich). During passage 5, cells were seeded onto gelatin-coated 48-well plates. After overnight incubation with glucose-free DMEM (Sigma-Aldrich), HCtAECs were supplemented with 1% heparin (Sigma-Aldrich) and 0.5% endothelial cell growth supplement (Sigma-Aldrich), and both HCtAECs and EA.Hy cells were supplemented with 10% FBS and 1% penicillin and streptomycin. Cells were then stimulated with or without 50 nM phorbol myristic acid or 50 μg/mL lipopolysaccharide in 5 or 30 mM glucose (Braun). sCD93 was measured as described above.

RESULTS

Plasma Levels of sCD93 in IMPROVE Study

In the IMPROVE Study, latitude was the strongest independent predictor of IMT (17). No significant association was observed between sCD93 and latitude (P = 0.942). Consistent with previous reports (11), there was no significant difference between men and women (mean ± SD: 162 ± 42 vs. 163 ± 45 ng/mL, P = 0.3833). Levels of sCD93 were significantly lower in subjects with T2D (157 ± 40 ng/mL) compared with those without T2D (164 ± 45 ng/mL, $P < 0.0001$). Thus, the cohort was stratified for diabetes status as this is likely to impact upon further analysis of IMT or other CVD risk factors.

sCD93 Levels and Metabolic or Cardiovascular Risk Markers

In the subjects without diabetes, sCD93 correlated with age, height, and metabolic markers (BMI, insulin, HOMA indices, vitamin D, and adiponectin) (Table 2). Consistent with lower levels being associated with poor metabolic control, sCD93 was positively correlated with adiponectin and vitamin D, but inversely with BMI, insulin, and HOMA. The association between sCD93 and lipids was confounded by lipid-lowering medication (Table 2). In lipid-lowering–naive subjects, sCD93 levels were associated with an advantageous metabolic profile (i.e., positively with HDL levels and

Table 2—Spearman rank correlation coefficients between sCD93 and cardiovascular risk markers

	Without T2D		With T2D	
	ρ	*P*	ρ	*P*
Sex	−0.001	0.9671	−0.045	0.1882
Age (years)	0.080	**0.0001**	0.166	**<0.0001**
Height (m)	−0.063	**0.0022**	−0.054	0.1163
BMI (kg/m^2)	−0.073	**0.0003**	−0.042	0.2199
WHR	0.023	0.2672	0.007	0.8511
SBP (mmHg)	−0.033	0.1067	−0.099	**0.0042**
SBP (mmHg)*	−0.023	0.4501	0.038	0.5048
DBP (mmHg)	−0.020	0.3319	−0.016	0.6428
DBP (mmHg)*	−0.050	0.0940	−0.003	0.9619
LDL cholesterol (mmol/L)	0.042	**0.0422**	−0.028	0.4210
LDL cholesterol (mmol/L)#	0.006	0.8265	0.048	0.3165
HDL cholesterol (mmol/L)	−0.073	**0.0003**	−0.081	**0.0195**
HDL cholesterol (mmol/L)#	0.056	**0.0343**	−0.019	0.6872
TG (mmol/L)	−0.051	**0.0131**	−0.010	0.7663
TG (mmol/L)#	−0.098	**0.0002**	−0.074	0.1106
Fasting glucose (mmol/L)	−0.014	0.5101	0.020	0.5704
C-reactive protein (mmol/L)	−0.014	0.5101	0.020	0.5704
Current smoking	0.022	0.2833	−0.004	0.9027
Lipid-lowering medication	−0.024	0.2455	0.000	0.9915
Antihypertensive medication	−0.025	0.2191	0.033	0.3467
Fasting proinsulin (pmol/L)	−0.025	0.2223	−0.024	0.4826
Fasting insulin (pmol/L)	−0.078	**0.0001**	−0.007	0.8293
HOMA-B	−0.054	**0.0076**	0.021	0.5333
HOMA-IR	−0.080	**0.0001**	−0.011	0.7263
Uric acid (μmol/L)	0.019	0.3567	0.023	0.5073
Creatinine (μmol/L)	0.173	**<0.0001**	0.237	**<0.0001**
Vitamin D (nmol/L)	0.067	**0.0009**	0.032	0.3310
Adiponectin (μg/mL)	0.063	**0.0022**	0.028	0.4159
Leptin (ng/mL)	−0.025	0.2197	0.000	0.9932
IL-5 (pg/mL)	0.091	**<0.0001**	0.034	0.3060
FRS	0.022	0.2719	0.103	**0.0019**

T2D was defined as diagnosis, antidiabetic medication, or fasting glucose level ≥7 mmol/L. FRS, Framingham Risk Score; vitamin D, adjusted for season of blood sampling. Boldface type indicates nominal statistical significance. *Subjects not receiving antihypertensive medication (n = 1,120 and 316 for subjects without diabetes and with T2D, respectively). #Subjects not receiving lipid-lowering medication (n = 1,426 and 462 for subjects without diabetes and with T2D, respectively).

negatively with TGs). A negative correlation was observed between sCD93 levels and SBP; however, this association was lost when analyzing subjects who had not received antihypertensive medication. Associations with metabolic variables remained significant after adjustment for age and sex (Supplementary Table 2).

sCD93 Levels and IMT in IMPROVE Study

Because cardiovascular risk factors have a large impact on IMT measures (16,17), these parameters were considered for inclusion in multiple regression models. Proinsulin and insulin measurements were omitted because they are not informative in subjects with diabetes (due to the influence of medication and pathology). Diabetes-stratified multiple-regression analysis gave rise to the following three models: *1*) age and sex; *2*) with variables significant in both subjects with and without T2D, where DBP, TG levels, creatinine levels, and current smoking status were added to model 1; and *3*) further inclusion of variables that were significant in one stratum (LDL, IL-5, adiponectin, and SBP). sCD93 was not associated with any baseline or progression measures of IMT in subjects with or without T2D, when adjusting for age and sex (Supplementary Table 3), or in the regression models adjusting for established CVD risk markers (data not shown). We could exclude lack of power as a reason for failing to detect an association (assuming

an effect size of ≥0.009 yielded a power of 0.99 for subjects without T2D, and 0.81 for the subjects with T2D). Thus, we conclude that sCD93 levels do not influence IMT.

T2D Risk–Associated SNPs and sCD93 Levels

A Mendelian randomization experiment was conducted to assess whether reduced sCD93 levels are a consequence or possible cause of T2D. If reduced sCD93 levels are a consequence of diabetes-related processes and/or susceptibility, then genetic variants that influence the risk of T2D would be expected to influence sCD93 levels. Genotypes of 53 (of 62) known (18) T2D risk–associated SNPs were available for the IMPROVE Study cohort and were analyzed for association with sCD93 levels (adjusting for age, sex, and population structure in subjects without diabetes). Individually, no SNP met the Bonferroni-corrected P value for significance ($P < 9.43E-4$) (Supplementary Table 4), nor was there any correlation with sCD93 levels for SNPs combined in an unweighted SNP score (Spearman rank correlation: $\rho = 0.0045$, $P = 0.8248$). These findings indicate that genetic susceptibility to T2D is unlikely to be a cause of reduced CD93 levels; hence, it is possible that reduced sCD93 levels precede the development of T2D.

sCD93 Levels in the Prospective SDPP Cohort

In order to assess whether the multiple metabolic aberrations that characterize the IMPROVE Study affect the results presented, the prospective SDPP cohort, specifically designed to assess potential biomarkers of T2D, was investigated. Baseline and follow-up features are presented in Supplementary Table 5 and Table 3, respectively. Baseline levels of sCD93 did not differ across glucose tolerance groups: NGT 163 ± 44 ng/mL, prediabetes 158 ± 44 ng/mL, and T2D 158 ± 41 ng/mL (ANOVA, $P = 0.23$, adjustment for age and sex). Similarly, no significant difference was found between follow-up levels of NGT, prediabetes, or T2D (153 ± 42, 154 ± 51 and 154 ± 48 ng/mL, respectively). To assess whether baseline sCD93 levels influenced the progression to prediabetes or to T2D over the time, baseline levels were compared between subjects (all NGT at baseline) who received a diagnosis of NGT, prediabetes, or T2D at follow-up. Subjects who remained NGT at follow-up had significantly higher baseline levels of sCD93 than those who progressed from NGT to T2D during follow-up (166 ± 44 vs. 158 ± 45 ng/mL, respectively; t test, $P = 0.016$). A similar nonsignificant trend of higher baseline sCD93 levels was observed in subjects who remained NGT at follow-up compared with those who progressed to prediabetes during follow-up (166 ± 44 vs. 161 ± 44 ng/mL, respectively; $P = 0.058$). Logistic regression demonstrated that baseline sCD93 levels were significantly associated with progression to poor metabolic control (prediabetes or T2D: β −0.612, SE 0.272, $P = 0.024$) but not to T2D specifically (β −0.573, SE 0.346, $P = 0.098$), and this was independent of current smoking status, use of blood pressure medication, and BMI (β −0.894, SE 0.298, $P = 0.003$). However, the inclusion of sCD93 levels in the model did not provide additional benefits (area under the receiver operating characteristic curve 0.73, irrespective of the inclusion of sCD93).

These results support the hypothesis that reduced sCD93 levels occur before the onset of T2D.

cd93-Deficient Mouse Model

A *cd93*-deficient mouse model, where there was no gross phenotypic abnormality, has been described previously (6). However, mice demonstrated reduced phagocytic activity (6), defective maturation of B cells and iNKT cells (23,25), and altered vascular permeability in glioma (9). These mice lack only exon 1 of the *cd93* gene and had a mixed genetic background (129/sv embryonic stem cells crossed to C57BL/6J). In contrast, our strategy maintained a genetically pure strain, namely C57BL6/N, with the entire *cd93* gene being deleted. This *cd93*-deficient mouse model again showed no gross phenotypic defect; however, there was partial lethality. Importantly, mice carrying one *cd93* gene ($cd93^{+/-}$) had approximately half the concentration of circulating scd93 compared with wild-type mice ($cd93^{+/+}$; 104 ± 18 vs. 254 ± 63 ng/mL, respectively; $P = 0.008$) (Supplementary Table 1). Compared with $cd93^{+/+}$ mice, $cd93^{+/-}$ mice showed no difference in mature B-cell populations (determined by the percentage IgG- or IgM-positive B cells) or iNKT cells (Supplementary Table 1). Therefore, these mice were appropriate for our studies aimed at investigating whether reduced levels of scd93 influence the development of atherosclerosis and T2D.

Atherosclerosis in $Apoe^{-/-}cd93^{+/+}$ Versus $Apoe^{-/-}cd93^{+/-}$ Mice

To investigate the impact of CD93 on atherosclerosis, the *cd93*-deficient mouse model was crossed with the $apoe^{-/-}$ mice, which are commonly used to study atherosclerosis. $apoe^{-/-}cd93^{+/+}$ and $apoe^{-/-}cd93^{+/-}$ mice were fed a chow diet until being sacrificed at 32 weeks. Although atherosclerotic lesions were visible in the descending aorta, there was no difference between $apoe^{-/-}cd93^{+/+}$ and $apoe^{-/-}cd93^{+/-}$ mice regarding the lesion area observed (Fig. 1). Thus, these data are consistent with the human findings that scd93 levels do not influence IMT.

Metabolic Characteristics of $cd93^{+/+}$ Versus $cd93^{+/-}$ Mice

To mirror the human metabolic findings, we investigated whether mice with reduced sCD93 levels had impaired glucose metabolism. When fed a chow diet, both genotypes demonstrated a similar rate of glucose clearance; however, $cd93^{+/-}$ male mice had higher basal levels of glucose compared with $cd93^{+/+}$ mice (mean ± SEM: 187.4 ± 13.6 vs. 161.9 ± 4.2 mg/dL, respectively, after fasting for 4 h) (Fig. 2). Female mice demonstrated no significant difference (mean ± SEM: 137.5 ± 4.7 vs. 137.4 ± 5.7 mg/dL, after fasting for 4 h) (Supplementary Fig. 2). However, when fed a Western diet (21% fat, 0.2% cholesterol), male $cd93^{+/-}$ mice demonstrated impaired clearance of glucose and reduced sensitivity to insulin

Table 3—Characteristics of the SDPP subjects receiving a diagnosis of NGT at baseline

Follow-up diagnosis	Without T2D	Prediabetes	With T2D	*P* value (ANOVA)
*N**	370	314	158	
Male (%)	200 (54)	179 (57)	110 (69)	**0.0019**
Baseline				
Age (years)	47.3 (4.7)	48.2 (4.4)	48.2 (4.6)	**0.0102**
Height (m)	1.73 (0.09)	1.72 (0.09)	1.74 (0.09)	0.1294
Weight (kg)	74.9 (12.4)	81.8 (14.1)	85.6 (15.2)	**<0.0001**
BMI (kg/m^2)	24.9 (3.2)	27.6 (4.1)	28.4 (4.7)	**<0.0001**
WHR	0.84 (0.07)	0.87 (0.07)	0.90 (0.06)	**<0.0001**
SBP	121 (14)	128 (15)	130 (15)	**<0.0001**
DBP	76 (9)	80 (9)	81 (9)	**<0.0001**
Fasting glucose (mmol/L)	4.6 0 (0.49)	4.94 (0.50)	5.06 (0.56)	**<0.0001**
Fasting insulin (mU/L)	14.2 (6.3)	17.5 (9.0)	21.2 (10.2)	**<0.0001**
sCD93 (ng/mL)	166 (44)	161 (44)	158 (45)	0.0700
Current smokers (%)	89 (22.2)	105 (29.2)	63 (36.8)	**0.0012**
BP treatment (%)	19 (4.8)	38 (10.6)	18 (10.6)	**0.0055**
Follow-up				
Follow-up time	9.1 (1.3)	9.2 (1.2)	9.5 (1.2)	**0.0025**
Age (years)	56.5 (4.8)	57.4 (4.5)	57.7 (4.7)	**0.0021**
Height (m)	1.72 (0.09)	1.71 (0.09)	1.73 (0.09)	0.0727
Weight (kg)	77.1 (13.3)	86.5 (15.9)	91.0 (18.2)	**<0.0001**
BMI (kg/m^2)	25.9 (3.4)	29.4 (4.8)	30.3 (5.8)	**<0.0001**
WHR	0.88 (0.06)	0.91 (0.06)	0.94 (0.07)	**<0.0001**
SBP	133 (17)	143 (17)	144 (18)	**<0.0001**
DBP	82 (10)	87 (10)	87 (11)	**<0.0001**
Fasting glucose (mmol/L)	4.86 (0.46)	5.72 (0.68)	7.35 (2.18)	**<0.0001**
Fasting insulin (mU/L)	14.6 (6.0)	21.2 (11.9)	26.6 (13.2)	**<0.0001**
sCD93 (ng/mL)	153 (42)	154 (51)	154 (48)	0.9654
ΔsCD93	13 (46)	7 (52)	4 (55)	0.0814
Current smokers (%)	61 (15.3)	73 (20.3)	34 (20.0)	0.1486
T2D treatment (%)	0	0	39 (22.8)	**<0.0001**
BP treatment (%)	64 (16.0)	133 (36.9)	72 (42.1)	**<0.0001**

Values are presented as the mean (SD) for continuous measures and *n* (%) for categorical measures, unless otherwise indicated. Prediabetes was defined as impaired glucose tolerance and/or impaired fasting glucose. ΔsCD93, baseline sCD93 − follow-up sCD93. Boldface type indicates nominal statistical significance. BP, blood pressure. *Smallest *n* for any variable.

compared with *cd93*$^{+/+}$ mice, which was not due to a difference in weight (Fig. 2). This was not seen in female mice (Supplementary Fig. 2). Levels of fasting insulin and biomarkers of metabolic dysregulation (leptin, glucagon, resistin, and glucagon-like peptide 1) were measured and compared between *cd93*$^{+/-}$ and *cd93*$^{+/+}$ mice (Table 4). Although not statistically different, a trend was observed whereby *cd93*$^{+/-}$ mice had increased levels of insulin and leptin levels compared with *cd93*$^{+/+}$ mice and were more insulin resistant (as measured by HOMA of insulin resistance [HOMA-IR]).

Assessment of Pancreas Morphology (*cd93*$^{+/+}$ vs. *cd93*$^{+/-}$)

The number and the average size of islets did not differ between *cd93*$^{+/+}$ and *cd93*$^{+/-}$ mice (21.7 vs. 24.1, $P = 0.34$ and 595 vs. 622 pixels, $P = 0.42$, respectively). Insulin staining was visible in islets in all genotypes; however, some interstitial insulin staining was apparent in sections from the *cd93*$^{+/-}$ mice (Fig. 3, top panel). As expected, VWF staining was restricted to the endothelium in all genotypes (Fig. 3, middle panel). In *cd93*$^{+/+}$ mice, cd93 demonstrated endothelial staining (as expected with cell surface–attached cd93) (Fig. 3, bottom panel) similar to that of VWF. Diffuse cd93 staining was also observed in the islets. Although this could reflect a previously unappreciated expression of cd93 by β-cells, we believe that it is more likely that the diffuse staining reflects the scd93 released from the endothelial cells. In *cd93*$^{+/-}$ mice, the endothelial cd93 staining was less obvious, but the diffuse cd93 staining was clearly visible. However, in the pancreas

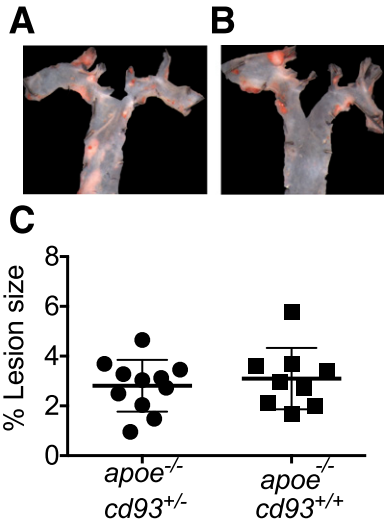

Figure 1—Representative image of the descending aorta stained with Sudan IV from *apoe*$^{-/-}$*cd93*$^{+/-}$ mice (*A*) and *apoe*$^{-/-}$*cd93*$^{+/+}$ mice (*B*). *C*: Quantification of lesions in the descending aorta of female and male *apoe*$^{-/-}$*cd93*$^{+/-}$ (black dots, n = 11 [6 male, 5 female]) or *apoe*$^{-/-}$*cd93*$^{+/+}$ (black squares, n = 9 [5 male, 4 female]) mice.

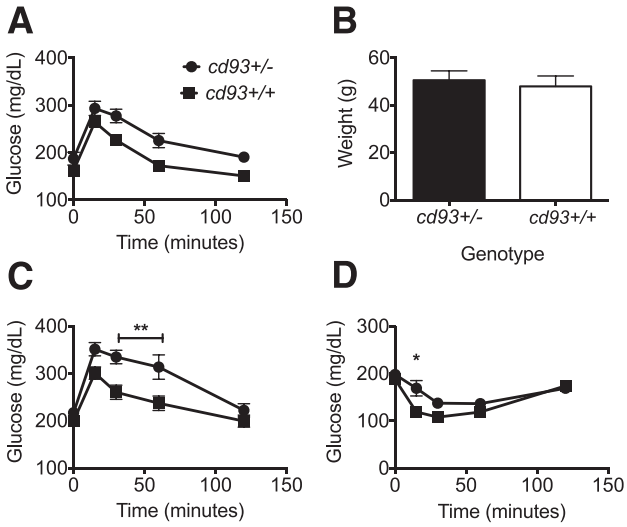

Figure 2—Glucose metabolism of *cd93*$^{+/-}$ male mice compared with *cd93*$^{+/+}$ male mice (black dots and black squares, respectively). *A*: Glucose tolerance test of *cd93*$^{+/-}$ and *cd93*$^{+/+}$ male mice (n = 10–13), 4 months of age, before being fed a Western diet. *B*: Weight of *cd93*$^{+/-}$ and *cd93*$^{+/+}$ male mice (n = 9–12, respectively) after 16 weeks of eating a Western diet. *C*: Glucose tolerance test of *cd93*$^{+/-}$ and *cd93*$^{+/+}$ male mice (n = 9–12, respectively) after 16 weeks of eating a Western diet. *D*: Insulin tolerance test of *cd93*$^{+/-}$ and *cd93*$^{+/+}$ male mice (n = 9–11 respectively) after 16 weeks of eating Western diet. Repeated-measures two-way ANOVA showed statistical significance for *C* and *D* with $^{**}P \leq 0.01$ or $^{*}P \leq 0.05$ statistical significance at a particular time point/s between genotypes using post hoc analysis of Šidák multiple-comparisons test. The error bar indicates SEM.

obtained from a *cd93*$^{-/-}$ mouse, no cd93 staining was observed. Interestingly, *cd93*$^{+/-}$ mice had a trend (P = 0.08) for a decreased percentage of VWF-positive islets compared with *cd93*$^{+/+}$ mice, indicating the presence of endothelial disturbances in Western diet–fed *cd93*$^{+/-}$ mice (Fig. 4*A*).

Pancreatic Blood Vessel Integrity

Given the role of cd93 in vessel leakage (9) and the interstitial insulin staining in *cd93*$^{+/-}$ mice, we performed an in vivo blood vessel permeability assay using Evans Blue dye. Under physiologic conditions, the endothelium is impermeable to albumin, so Evans Blue–bound albumin remains confined within blood vessels. The presence of Evans Blue dye within a tissue after perfusion with PBS indicates leakage out of blood vessels into the interstitial space. Interestingly, young *cd93*$^{+/-}$ mice had an increase in Evans Blue dye compared with *cd93*$^{+/+}$ littermates (Fig. 4*B*). The finding that higher levels of Evans Blue dye were detected in *cd93*$^{+/-}$ mice than in *cd93*$^{+/+}$ mice provided confirmation that the (albeit weak) interstitial insulin staining in the pancreata of *cd93*$^{+/-}$ mice was not merely an artifact. Thus, lacking cd93 even at a young age results in leaky vessels; however, neither young animals nor mice fed rodent chow displayed a diabetes phenotype. Therefore, we questioned whether after the metabolic stress of a Western diet there were signs of endothelial damage in plasma, indicated by the presence of soluble e-selectin and VWF A2. Indeed, *cd93*$^{+/-}$ mice fed a Western diet demonstrated an increase in both endothelial damage markers compared with *cd93*$^{+/+}$ mice (Fig. 4*C* and *D*), indicating endothelial damage in the Western diet–fed *cd93*$^{+/-}$ mice.

Influence of High Glucose Levels on Release of sCD93 From Endothelial Cells

Because diffuse cd93 staining was observed in islets, and because metabolic regulation in mice was impaired after dietary stress, we investigated whether the release of sCD93 (by known stimuli) might be influenced by hyperglycemia, mimicking the prediabetes state. Glucose

Table 4—Peripheral fasting levels of diabetes relevant analytes

	cd93$^{+/-}$	*cd93*$^{+/+}$	*P* value
Glucose (nmol/L)	12.6	11.1	**0.03**
Insulin (ng/mL)	17.4	12.6	0.24
Leptin (ng/mL)	62.2	51.5	0.24
Resistin (ng/mL)	164	183	0.30
Glucagon (ng/mL)	0.09	0.07	0.50
GLP-1 (ng/mL)	0.03	0.01	0.26
HOMA-IR*	0.25	0.16	0.15
Total cholesterol (mg/dL)	442	433	0.86
TG (mg/dL)	138	139	0.89

GLP-1, glucagon-like peptide 1. Boldface type indicates nominal statistical significance. *HOMA-IR was calculated by G0 × I0 /22.5, where I0 is fasting blood insulin (μU/mL) and G0 fasting blood glucose (mmol/L).

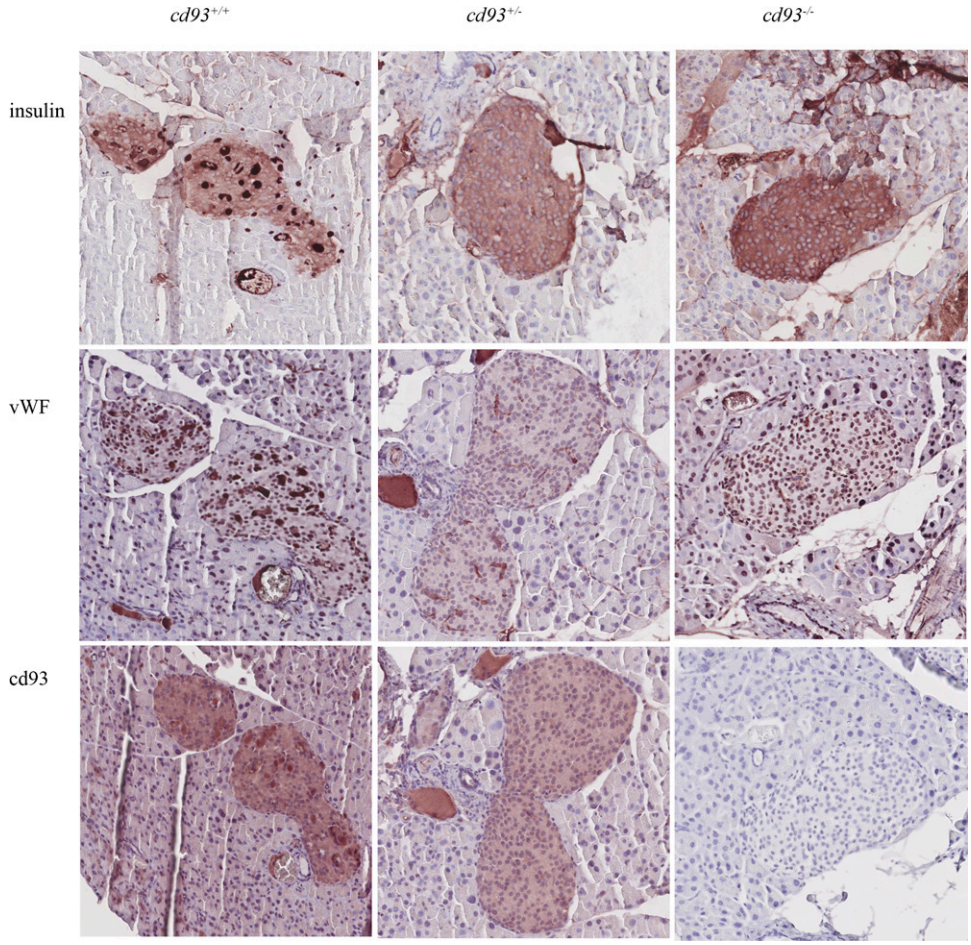

Figure 3—Immunohistochemistry of pancreas sections demonstrating the location of insulin, sCD93, and VWF in mice with 2, 1, or 0 copies of the *cd93* gene ($cd93^{+/+}$, $cd93^{+/-}$, and $cd93^{-/-}$, respectively).

levels did not influence the release of sCD93 from primary HCtAECs under basal (media) or lipopolysaccharide-stimulated conditions (Supplementary Fig. 3*A*); however, hyperglycemia (30 mM glucose) reduced phorbol myristic acid–stimulated release of sCD93 compared with normoglycemia (5 mM glucose). This experiment was repeated with the EA.Hy 926 cell line, with comparable results (Supplementary Fig. 3*B*).

DISCUSSION

The main objective of this study was to elucidate whether sCD93 plays a role in metabolic disease or CVD. Our results refute sCD93 as an important factor in the early vascular changes indicative of atherosclerosis; however, they do provide solid evidence for a role of sCD93 in glucometabolic regulation and a starting point for understanding the role of CD93 in these diseases.

The most striking result from the current study is the finding that reduced levels of sCD93 were associated with metabolic dysregulation. In addition, we show the following: *1*) lower sCD93 levels were observed in subjects with T2D who had high CVD risk than in those subjects without T2D; *2*) insulin-related processes were associated with sCD93 levels in subjects without diabetes; *3*) lower levels of sCD93 were not due to genetic susceptibility to T2D; *4*) lower sCD93 levels precede the development of T2D; *5*) dietary stress in a *cd93*-deficient mouse model caused impaired metabolic regulation and increased endothelial damage; *6*) cd93 (both cell surface bound and soluble) was detected in islets; and *7*) hyperglycemia impaired the release of sCD93 by specific stimuli. The lack of association between sCD93 levels and early atherosclerosis measures is consistent between human and mouse.

Thus, we propose that CD93 expression and sCD93 release in pancreatic islets are components of stress responses and are important for endothelial integrity and thereby metabolic control. CD93 deficiency leads to leaky blood vessels, which under normal metabolic conditions is tolerated or compensated for. However, when stressed (inflammatory or metabolic), the release of sCD93 is further impaired, possibly leading to endothelial damage. Leaky vessels and endothelial damage would permit insulin diffusion into the interstitial space, leading to suboptimal insulin delivery to distal tissues. These results are the first direct evidence for the recently proposed role for CD93 in T2D (12).

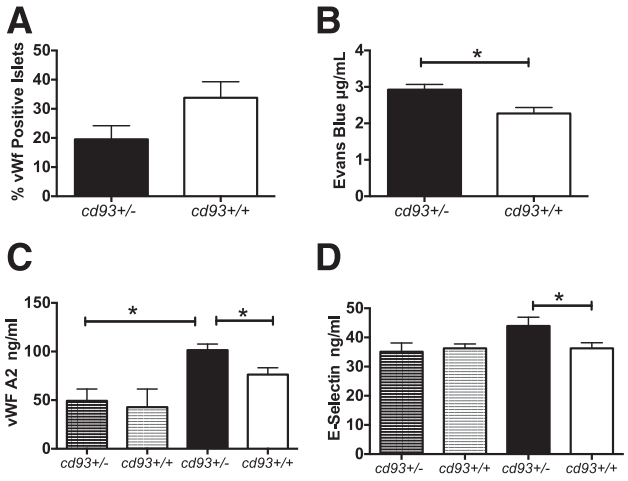

Figure 4—Vascular integrity and endothelial damage in *cd93*$^{+/+}$ and *cd93*$^{+/-}$ mice. *A*: Percentage of VWF-positive islets in pancreata from mice fed for 16 weeks with a Western diet (four of each genotype). *B*: Quantification of Evans Blue dye in pancreata in *cd93*$^{+/-}$ and *cd93*$^{+/+}$ mice (four of each genotype; black and white bars, respectively). *$P \leq 0.05$, Student *t* test. *C*: Plasma levels of soluble VWF A2 in *cd93*$^{+/-}$ and *cd93*$^{+/+}$ mice that were fed for 16 weeks with a Western diet (black and white bars, respectively) or a chow diet (dark and light hashed bars, respectively), n = 9–11 per genotype, *$P \leq 0.05$, one-way ANOVA, using Tukey multiple-comparison test between genotypes and diet. *D*: Plasma levels of e-selectin in *cd93*$^{+/-}$ and *cd93*$^{+/+}$ mice that were fed for 16 weeks with a Western diet (black and white bars, respectively) or a chow diet (dark and light hashed bars, respectively), n = 9–11 per genotype. *$P \leq 0.05$ Student *t* test. All error bars indicate the SEM.

In view of previous publications on CD93, it should be noted that there was no evidence to suggest that changes in iNKT cells were responsible for the effects reported here, in contrast with previous reports (10). In addition, two SNPs associated with sCD93 levels in control subjects have been described (11). No associations were observed between these SNPs and sCD93 levels or insulin sensitivity (Supplementary Table 6) in the IMPROVE Study, nor do they demonstrate any association with T2D (DIAbetes Genetics Replication And Meta-analysis [DIAGRAM] Consortium, n = 100,589; rs2749812, P = 0.940; rs3746731 P = 0.870) (18). Although the IMPROVE Study is the largest cohort to date with data on sCD93 levels, this cohort is not metabolically uniform, in contrast to the myocardial infarction case patients and healthy control subjects (11), where few subjects were receiving lipid-lowering or antihypertensive medication and very few subjects had T2D. Therefore, comparisons between data from the study by Mälarstig et al. (11) and the IMPROVE Study should be approached with caution. We admit that the size of the SDPP replication study is limited; however, the use of an oral glucose tolerance test to define glucose control categories and the length of follow-up compensate for the restricted sample size. A further caveat is that the murine model demonstrated a sex difference that was not seen in the clinical data. The murine studies were conducted in mice of reproductive age; thus, it is plausible that age-related differences in hormones might contribute to this discrepancy. Women in the IMPROVE Study were all postmenopausal; thus, this effect was not seen. The SDPP cohort was younger, so it cannot be assumed that female participants in SDPP are postmenopausal; however, the size of the cohort precluded the assessment of sex-specific effects.

Previously, the release of sCD93 has been implicated as a response to stressors such as inflammatory, immune, and angiogenic mediators. Our demonstration of clear cd93 staining in pancreatic islets is novel and might reflect a protective function, whereby a deficiency in cd93 results in morphological and physiological changes in the pancreas. Furthermore, the in vitro studies showing that sCD93 was not released from endothelial cells as efficiently under hyperglycemia fits with the documented downward spiral of glycemic control that is characteristic of T2D progression.

Differences in sCD93 levels between subjects with and without diabetes are subtle; therefore, it is unlikely that the measurement of sCD93 levels would have clinical utility as a biomarker. However, given that this molecule might mediate both inflammatory and metabolic pathways, further investigation and understanding of CD93 functions is warranted and might provide opportunities for future preventive strategies. Having established the *cd93*-deficient mouse model and confirmed the human relevance, we are able to continue to conduct a deeper functional evaluation of cd93.

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Author Contributions. R.J.S. designed and conducted study, measured and analyzed sCD93, managed and performed quality control of phenotypic and genetic data for IMPROVE, conducted the immunohistochemistry, and drafted the manuscript. A.Hil. and C.-G.Ö. collected and phenotyped the Stockholm Diabetes Prevention Program cohort. A.S. measured and analyzed sCD93. C.Ö. conducted the immunohistochemistry. B.S. managed and conducted the quality control of phenotypic and genetic data for the IMPROVE Study. O.M. assisted in statistical analysis of the IMPROVE cohort. P.T. and F.F. measured and analyzed sCD93 and were responsible for the animal studies. E.T., D.B., F.V., R.R., A.J.S., P.G., S.K., E.M., E.G., S.E.H., and U.d.F. conducted the IMPROVE cohort collection and phenotyping. A.-C.S. oversaw the genotyping. L.M. was responsible for the animal studies. A.Ham. conducted the IMPROVE cohort collection and phenotyping and was responsible for the animal studies. A.B. designed and conducted the study, measured and conducted the analysis of soluble CD93, was responsible for the animal studies, conducted the immunohistochemistry, and drafted the manuscript. All authors edited and approved the manuscript. R.J.S. and A.B. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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